PLATE XIII.

Fig. 35. Bristle of the 1st foot of *Onuphis quadricuspis*, Sars. It is accidentally curved. × Zeiss oc. 2, obj. D.
Fig. 36. 20th foot of the same. Enlarged.
Fig. 37. Powerful winged and bifid hook from the posterior region of the same. × as before.
Fig. 38. Bristle of the 1st foot of *Onuphis* near *holobranchia*, Marenzeller. × as before.
Fig. 39. 10th foot of the same. Magnified.
Fig. 40. Foot (about 20th) of *Eunice* from Cape Rosier. Enlarged.
Fig. 41. Ventral bristles of the same. × as before.
Fig. 42. Upper bristle of *Lumbriconereis assimilis*. × as before.
Fig. 43. Winged hook of the same. × as before.
Fig. 43 *a*. Head and anterior end of the foregoing. Enlarged.
Fig. 44. Anterior end of *Drilonereis canadensis*. Enlarged.
Fig. 45. Upper bristle of the same. × as before.
Fig. 46. 50th foot of the same. × 70 diam.

VII.—*On some Points in connexion with the ordinary Development of* Vaucheria *Resting-spores*. By H. CHARLTON BASTIAN, M.A., M.D., F.R.S., F.L.S.

[Plate XIV.]

COMPARATIVELY few persons have probably followed the development of the resting-spores of *Vaucheria*, owing to the length of time they remain in a dormant condition. I have long been familiar with these bodies and with various changes that are from time to time apt to occur therein, but until the summer of 1901 I had never seen them germinate and give rise to young *Vaucheria* plants.

The only description of their germination that I have been able to find is that given by Pringsheim *. He says:—"The spore remains for some time longer, without being thrown off from the parent tube on which it was produced; but the colour of its contents, which was at first green, gradually becomes paler and paler; the spore is at last rendered quite colourless and presents in its interior only one or more largish dark brown bodies. When it has lost all its colour it is detached from the parent tube in consequence of the decay of the membrane of the sporangium enclosing it. After some

* "On the Impregnation and Germination of Algæ." Translation in Quart. Journ. Microsc. Science, 1856, p. 63, pl. iii. figs. 17–20. The figures, as reproduced in the Journal, and as copied in Cooke's 'British Freshwater Algæ,' are very crude, and even erroneous in several respects; this is especially the case with fig. 17.

time (in my experiments after about three months) the spore, which is readily recognizable by the red-brown nuclei in its interior, suddenly resumes its green colour, and immediately thereupon grows into a young *Vaucheria,* exactly resembling the parent plant. Close observation shows that the innermost layer, elongating, breaks through the thick outer membrane and becomes the young tube exactly in the same way as I have described the process of development in the germinating spore of *Spirogyra.*"

On May 8th, 1901, I had under examination a quantity of one of the larger *Vaucheria* which had been gathered a fortnight previously and placed with water in a shallow dish. Much of the weed had died, but on and among the filaments I found a very large number of resting-spores. A quantity of these were on this day placed in a small wide-mouth bottle, loosely covered with a screw cap, merely to exclude dust and diminish the amount of evaporation. The bottle was half-filled with water, and was then left, not far from a window, on the end of a mantelpiece in my study.

The bottle was at first opened only on two or three occasions for a brief examination of its contents. The spores were soon found to be undergoing the common kind of change —that is, were becoming decolorized into a whitish-grey mass of granules and vesicles, containing in its midst from one to four aggregations of pigment-granules. The pigment-heaps in this case were of a reddish-brown or reddish-orange colour, though very frequently the tint is found to be of a blackish green.

After an interval of several weeks, on July 4th, I examined the contents of this bottle again, and in the first portion of the deposit taken up with a pipette I found a number of the resting-spores germinating and giving birth to filaments. They were associated with other spores in their ordinary condition and others still in which different changes had been taking place.

Both the resting-spores themselves and the filaments that had grown from them were lined, sometimes pretty thickly and at others very sparsely, with bright green chlorophyll-corpuscles. In regard to the filaments, the most common arrangement was that the single process sent out almost immediately divided into two at a very obtuse angle; at other times the division took place at some distance from the spore, while occasionally two filaments were seen coming off from the spore itself close to one another. Subsequently they branched and changed in diameter in a very irregular manner.

The most remarkable facts, however, about these germinating resting-spores have had reference to the contained heaps of pigment, loosely spoken of in the foregoing translation from Pringsheim as "nuclei." The facts observed were so remarkable that I was anxious to repeat my observations in the following year, and this was done.

On June 8th, 1902, I found a quantity of *Vaucheria racemosa*, growing at the edge of a pond, which was absolutely crowded with resting-spores. Some of this plant was kept in water in a shallow dish, and after a day or two numbers of the spores were placed in a small stoppered bottle half-full of water. Another large quantity of spores was placed with water in a tumbler, and merely covered loosely, so as to exclude dust.

On July 24th—that is, only six and a half weeks from the date of gathering—many of the resting-spores within the stoppered bottle were found to have germinated, though at that date none of the spores in the tumbler could be found in this condition. Subsequent examinations made it clear that germination took place more rapidly in the closed bottle than in the open tumbler. I have no record of the date when I first found them germinating in the tumbler, but I can say that at the expiration of three and a half months hundreds of these spores were germinating, and that the process was seen occurring in others of them during the next two months.

It may not be out of place to say a few words now concerning the condition of these resting-spores in the long interval that occurs between decolorization and germination.

For a long time they remain, without apparent change, of a greyish-white colour, owing to the presence of an intimate mixture of colourless granules and corpuscles, the latter seeming to be derivatives or remainders from the green corpuscles with which the spores were originally packed. More or less in the centre a large mass of pigment-granules, mostly of a blackish-green colour (Pl. XIV. fig. 1, A, × 250), but sometimes of a red-brown or red-orange tint, as in *V. racemosa*, is to be seen. Three or four, or even more, smaller pigment-heaps, instead of one large one, are very common in this latter species. The largest number I have ever found is shown in B (× 250). It seems perfectly clear that these heaps of finely granular pigment are merely refuse products left over during the process of molecular transformation that the spore has undergone in becoming decolorized. Microscopical examination shows that they are mere heaps of fine granules, unsurrounded by any bounding membrane.

Now comes the question, what changes are undergone by the resting-spore in its ordinary condition, such as is shown in A, previous to its germination? It is very difficult to be certain as to this, but my impression, formed after the examination of very large numbers of these bodies, is that in the normal course of development the corpuscles indicated in A almost completely disappear and that the general substance of the spore becomes resolved into a uniform mass of very minute granules. The spore has then a rather glistening silvery-white appearance, such as was seen in B. In the specimen represented in C there was much the same silvery-white appearance, but there were indications that a new set of rather smaller spheres was forming, leading on to the production of small glistening spheres of protoplasm such as are shown in D. Thereafter these small spheres seem to become green and converted into small chlorophyll-corpuscles. A specimen of this kind is shown in E, but in an early stage, as the corpuscles were still only of a very pale green colour. Pringsheim speaks of "the innermost layer elongating," and says it "breaks through the thick outer membrane, and becomes the young tube." These two layers are distinguishable in E, which is, I believe, a spore becoming green and just about to germinate, while in fig. 3, D, the split in the outer membrane is recognizable.

Pringsheim further intimates that these latter changes are rapidly brought about. He says, after a time the spore "suddenly resumes its green colour, and immediately thereupon grows into a young *Vaucheria* exactly resembling the parent plant." As to the rapidity of these latter processes, my own observations do not enable me to make any definite statements.

I now come to the most interesting and important point of all in connexion with the germination of the *Vaucheria* resting-spore, namely, as to the fate of the pigment-heaps which all along have been such prominent objects in the resting-spores. During my first examination of these germinating spores and on all subsequent occasions I have found either in the green spore itself or in one of the filaments issuing therefrom one or more of the blackish-green or red-brown pigment-heaps now appearing (when not pressed together or squeezed within a filament) as perfect spheres with sharply defined outlines, such as are shown in fig. 2 (× 250). The most surprising thing at first was to see these pigment-spheres in the filaments, as in D and in E. The latter body is only enlarged half as much as the others, in

order to show the three unequal pigment-masses far away from the spore itself, and jammed together in the filament *. Many of the spheres have been seen very much further away in the filaments than this, and many also have been seen just emerging from the spore, as in C, where the middle body is much compressed between the other two.

To casual observation these bodies all appear to be motionless; but after exposure for a little while to the light and heat from the microscope-lamp, very faint and more or less imperceptible movements can be detected in most of them. During my first study of these bodies, while examining a spore containing two of them, as in B, I saw one of the spheres moving forwards and backwards over a space scarcely equalling its own breadth without any visible change in shape, and with a slow gliding movement like that of an *Actinophrys*. But through the walls of the spore no rays of any kind could be detected then or since, though I have repeatedly watched their very slow movements taking place when these spheres have been within the spores and also within the filaments. Sometimes there has been a distinct interval between the forward and the backward movements; at other times it is clear that the movements of the pigment-spheres must be more continuous in one direction.

In October last I had under observation a spore in which within and just outside there were four unequal pigment-spheres, and also two others some distance away within the filament. This specimen is shown in Pl. XIV. fig. 3, A (× 125), though the two spheres close together in the filament are very indistinct, owing to their being out of focus. After I had taken this photograph, with a short exposure, I noticed that the movements of the spheres within and near the spore were more marked than usual. I left the specimen therefore exposed to the light and heat of the lamp (I was using no screen at the time) for exactly fifteen minutes, and then I took the photograph represented in B (× 250), with an exposure of three minutes. The results shown are most interesting. During the fifteen minutes' interval the lowest sphere had evidently moved considerably, though from the sharpness of its outline it is clear that it can scarcely have moved at all during the taking of the photograph. The small upper sphere had evidently moved less, though very perceptibly, during the interval, and had oscillated during the three minutes in which the photograph was being taken, as its

* One of the two smaller bodies is flattened against the upper wall of the filament.

outline is so hazy and indistinct. The other small and the large sphere had moved comparatively little.

A rather large sphere of red-orange colour which was a long way out in a filament is shown in C (× 375) after it had been killed by a weak solution of formalin. The sharpness of its outline shows that all movements had been stopped. In some of the large spheres a very rudimentary development takes place. They no longer have the appearance shown in C; they seem to have grown somewhat, since around the heap of pigment-granules there is a rim of brownish-yellow protoplasm, such as is to be seen in D (× 250). This body is situated in an empty spore, whose outer membrane shows the rupture produced during germination.

On a single occasion only have I seen one of these pigment-spheres encysted. It was situated outside a filament from which it had been liberated *. This specimen was in the first stock of these germinating resting-spores that was examined, and was found after the spores had been in my possession in a small bottle for five months. The cyst showed a rough tuberculated margin as in E (× 500), and the contents were of a blackish-green colour. Although this body was outside the filament, there was no room for doubt as to its nature.

I have now examined two or three hundred germinating resting-spores of *Vaucheria*, and in every one of them the original pigment-heaps have been seen in one or other of the conditions just described—each of them, that is, has been found to be included in a small mass of protoplasm which has been formed around it in some way during the stages immediately preceding the germination of the spore. So long as the spore has not sent forth any filaments we see more or less ill-defined aggregates of pigment-granules, this being the case even up to the stage almost immediately preceding germination, such as is shown in Pl. XIV. fig. 1, D and E. On the other hand, as soon as germination has taken place we find these pigment-heaps, spherical, sharply defined, enclosed within a scanty amount of protoplasm, and exhibiting slight powers of independent movement, which, as with other low organisms, are destroyed by weak solutions of formalin or osmic acid.

There cannot be a doubt, in fact, that we have to do with

* The liberation is easily accounted for, as it very commonly happens that after a time the spore itself and one or more of the proximal segments of the filament die. All the chlorophyll-corpuscles of such segments disappear, while the membranes often become soft and disintegrated. The formation of dissepiments in both young and old filaments of *Vaucheria* is by no means uncommon.

the generation within the resting-spores of *Vaucheria* of independent forms of life of a very low order, resembling *Amœbæ* or the simplest forms of *Actinophrys*, but forms of life which are so heavily freighted with indigestible matter as to give them but a poor chance of undergoing further development.

March 20, 1903.—During the last three or four days I have again examined many specimens taken from the second batch of developed *Vaucheria* resting-spores contained in the open vessel. These examinations have been made after a long interval, and during this time much of the water had evaporated. Though rather more than ten months had elapsed from the date when the resting-spores were gathered, many of them were found to be still undeveloped. They were mixed with much débris from dead filaments, with empty cases of resting-spores, and with a large quantity of pale green *Vaucheria* filaments emanating from resting-spores which had germinated. Some of these filaments were still in continuity with, though others were separated from, the spores from which they had issued.

Each of the resting-spores in connexion with living filaments contained one or more of the pigmented Amœboid spheres. These were now found to be almost motionless, and none of them had wandered out into the green filaments. They were therefore probably some of the resting-spores that had recently developed, after long confinement under unfavourable conditions, with the result that the pigment *Amœbæ* were less active than those which had been produced at an earlier period.

On the other hand, many of the spores and filaments were dead, and from them all, or almost all, the chlorophyll-corpuscles had disappeared, though these filaments contained one, two, or more of the spherical *Amœbæ*, and many of them were in a more fully developed condition than any I had previously seen. The specimens had lapsed into a resting-stage and were perfectly motionless; but they were seen to possess a wider and more distinct border of protoplasm, stained of a slightly brownish colour, but free from pigment-granules. Some of these specimens, as in Pl. XIV. fig. 4, A (× 375), showed clear indications of a commencing segmentation of this peripheral protoplasm, while in others, as in B (× 375), segmentation had actually occurred into a number of minute monads, whose movements had to be arrested with a dilute solution of osmic acid before the photograph could be taken. Two or three of these monads (rather out of focus) may be

seen just escaping after rupture of the limiting membrane of the sphere.

In other specimens the change that occurred in these more developed pigment *Amœbæ* was different. No peripheral pigment-free protoplasm was developed, but a central nuclear mass of protoplasm, such as may be seen in C and D (× 375), was produced.

All these specimens, as I have said, were found in otherwise empty filaments—that is, in filaments denuded of chlorophyll-corpuscles. It is probable therefore that they were relatively old and were relicts of some of the earlier germinations.

I have only found a single specimen of the pigment *Amœbæ* in one of these more developed states while still contained within an old resting-spore. This specimen is shown in fig. 4, E (× 375), and it may be seen to represent a rather abortive attempt at the formation of one of the central nuclear masses.

Both these changes in resting *Amœbæ* are very familiar to me. They occur frequently in some other kinds of large *Amœbæ* which are apt to swarm in cells of *Nitellæ* and in *Vaucheria* filaments when these plants have been kept for a short time under certain unnatural conditions. These particular *Amœbæ*, however, grow most rapidly, while gorging themselves with chlorophyll-corpuscles. They then pass into a resting condition, and in the course of twenty-four hours or less many of them begin to segment peripherally into flagellate monads, while others, lying side by side with them, and therefore under similar conditions, for some mysterious reason go through the alternative process—that is, each of them gives rise to a single central sphere of protoplasm, which becomes surrounded by a membrane, and then remains in a quiescent condition for three or four months. After this long period the substance of the central mass of protoplasm also undergoes simultaneous segmentation into a number of minute flagellate monads or zoospores *.

It seems clear, therefore, that the sluggish *Amœbæ* whose origin has been traced from the mere heaps of pigment-granules always present within the resting-spores of certain species of *Vaucheria* tend, after comparatively long periods, to pass through developmental phases of a kind so definite as to remove all doubt as to the fact of their being independent

* These changes in *Nitella* and *Vaucheria* will be fully described in Part IV. of my 'Studies in Heterogenesis,' to be published about December next.

animal organisms, even though they have taken origin from the substance of the plant.

EXPLANATION OF PLATE XIV.

Fig. 1. A. An ordinary decoloured *Vaucheria* resting-spore.
B. A resting-spore of *V. racemosa* with many pigment-heaps and the substance generally composed of fine granules.
C. A spore very much like the last, but in which vesicles seem beginning to form again from the granular substance.
D. Another spore, in which the new vesicles are small and distinct, but colourless.
E. A spore almost ready to germinate, in which the small vesicles have taken on a pale green colour and the pigment-mass has become more defined in outline.
All × 250.

Fig. 2. A. A spore which has germinated lined with small chlorophyll-corpuscles and containing a single pigment-organism.
B. A spore with two pigment-organisms and scarcely any chlorophyll-corpuscles.
C. A spore with three pigment-organisms making their way out into the filament.
D. A spore and filament, showing a large pigment-organism in the latter compressed into an ovoid shape.
E. Another germinated spore, showing a large and two small pigment-organisms far out into the filament and compressing one another, one being flattened against the upper border of the filament.
A, B, C, D, × 250; E, × 125.

Fig. 3. A. A spore thickly lined with chlorophyll-corpuscles and containing four unequal pigment-organisms.
B. The same spore, more highly magnified, after an interval of fifteen minutes, in order to show indications of the movements of the organisms during the interval, during the period when this photograph was being taken, or during both these periods.
C. A large pigment-organism, far out in a filament well lined with chlorophyll-corpuscles, after it had been killed by a solution of formalin.
D. A more developed pigment-organism within an old spore, which, as well as the filament, was devoid of chlorophyll-corpuscles.
E. One of the pigment-organisms which has become encysted outside a filament.
A, × 125; B, × 250; C, × 375; D, × 250; E, × 500.

Fig. 4. A. Two pigment *Amœbæ* in an otherwise empty filament, with a distinct margin of brownish protoplasm, showing some indications of approaching segmentation.
B. One of the *Amœbæ* in which peripheral segmentation into monads has taken place.
C & D. Two pigment *Amœbæ* in which a central nuclear mass of protoplasm has been formed.
E. An old resting-spore, containing a pigment *Amœba* in which there is an abortive attempt at the formation of a similar nuclear mass of protoplasm.
All × 375.